SMEAR LAYER OUTCOME ON HEALING

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A thesis submitted to the Faculty of the
Endodontics Graduate Program
Naval Postgraduate Dental School
Uniformed Services University of the Health Sciences
in partial fulfillment of the requirements for the degree of
Master of Science
in Oral Biology

July 2015

Naval Postgraduate Dental School
Uniformed Services University of the Health Sciences
Bethesda, Maryland

CERTIFICATE OF APPROVAL

MASTERS THESIS

This is to certify that the Master's thesis of

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31 July 2015

NAVAL POSTGRADUATE DENTAL SCHOOL
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2015

ABSTRACT

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INTRODUCTION: The mechanical process of shaping the root canal creates a layer of organic and inorganic debris, termed the smear layer. This layer can be removed using a combination of ethylene-diamine-tetraacetic-acid (EDTA) and sodium hypochlorite (NaOCl). Smear layer removal is not the standard of care and its removal is debated. Currently, there are limited *in-vivo* endodontic outcome studies to aid the clinician's decision in the removal of the smear layer prior to obturation. **PURPOSE:** This continuing prospective, randomized, double-blinded clinical trial compared the endodontic outcomes of initial non-surgical root canal treatment of teeth with the smear layer removed compared to teeth where the smear layer was left intact. Secondarily, the influence of covariant factors on endodontic outcomes was analyzed.

METHODS: After initial comprehensive endodontic evaluation subjects were randomly assigned one of 2 groups. A standardized treatment protocol was followed with the exception of a final irrigation solution rinse. Sixty-eight subjects received 1ml/canal of 0.9% saline. Sixty-three received 1ml/canal of 17% EDTA followed by 3ml/canal of NaOCl as the final irrigant. The canals were then obturated. All subjects were recalled for a follow-up clinical and radiographic examination conducted no less than one year after treatment to determine the

endodontic outcome. Based on clinical and radiographic data, subjects were identified as healed or non-healed. The data were analyzed using the Fisher's Exact test (α=0.05). **RESULTS**: Interim analysis revealed no significant difference between irrigation protocols *(p=0.114)*. A secondary analysis revealed no covariant factors affected endodontic outcomes.

CONCLUSION: Under the conditions of this randomized, double-blinded *in-vivo* clinical study, smear layer removal did not affect endodontic outcomes.

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EDTA	ethylene-diamine-tetraacetic-acid
NaOCl	sodium hypochlorite
μm	micrometers
SEM	scanning electron microscopy
CT	computed tomography
PA	periapical

Chapter I: Introduction

A smear layer of dentin particles, tissue remnants, and bacteria is created during endodontic mechanical debridement of the root canal system. A combination of ethylene-diamine-tetraacetic-acid (EDTA) and sodium hypochlorite (NaOCl) has been shown to effectively remove the smear layer (Calt, Serper, 2002; Crumpton, Goodell, McClanahan, 2005). Removal of the smear layer is not universally practiced. To date, no *in-vivo* clinical study has been conducted to determine whether removal of the smear layer will result in a better endodontic outcome for permanent teeth.

Chapter II: Review of Literature

The Smear Layer

Cleaning and shaping the root canal system through the use of mechanical instrumentation, an integral part of non-surgical root canal treatment, produces a superficial layer of organic and inorganic substances against the canal wall and extending into the dentinal tubules (Torabinejad *et al.*, 2002). This layer, the smear layer, has the potential to also contain bacteria and their by-products. Empirically, many clinicians believe that removing the smear layer will result in better endodontic outcomes. There are however no clinical trials in permanent teeth to support this premise (Violich and Chandler 2010).

Smear Layer Characteristics

The smear layer was first identified by Eick *et al.* in 1970 on cut surfaces of tooth crowns using an electron microprobe with scanning electron microscopy (SEM). A smear layer in the root canal system was first described by McComb and Smith in 1975. They reported that "most standard instrumentation techniques produced a canal that was smeared and often packed with debris". Hand and rotary instrumentation generated a tenacious layer of closely compacted dentin particles and pulp tissue. When viewed under SEM it appears amorphous, irregular and granular (Sen *et al.* 1995).

According to Mader *et al.* (1984) the smear layer in a root canal is approximately 1 to 2 µm thick and penetrates into the dentinal tubules. Mjor and Nordhal (1996) observed dentinal tubules using light and SEM. They reported tubule densities ranging from 9 to 24 per 100µm (counted across the long axis of the tubules on demineralized, stained sections of 13 different teeth). The highest densities were found the coronal portion of teeth and closer to pulp tissue.

Density was lowest in the apical region and decreased toward the periphery. The tubules ranged from 1 to 3μm in diameter allowing bacterial penetration. Major and minor tubule branches ranged from major 0.5 to 1μm and 50 to 100nm in diameter respectively. Other investigators have reported finding diameters of 2.5-3.0 μm in deep dentin tubules allowing them to house smear layer particles up to a depth of 40 μm (Mader *et al.* 1984, Pashley 1992).

Bacterial Presence

In the landmark study comparing dental pulp exposures in germ-free and conventional rats by Kakehashi *et al.* (1965), devitalized pulps, apical granulomas and abscesses were only found in the conventional rats. Dentinal bridging and the absence of pathosis were observed in the germ-free animals indicating that the microbial flora was the main factor in the healing of exposed pulp tissue. This study proved that pulp necrosis was due to bacterial infection. Since the goal of endodontic treatment is to eliminate bacteria in the root canal, the presence of bacteria in a smear layer may impact the healing of apical pathosis.

Should the Smear Layer be Removed?

There currently is no clear consensus in the endodontic community as to whether the smear layer should, or should not, be removed prior to obturation. A survey by Moss *et al.* (2001) revealed that only 51% of endodontists and just 24% of dental students were removing the smear layer. Dutner *et al.* (2012) conducted another survey 11 years later asking the same question. This more current survey, published three years ago, reported that 77% of endodontists remove the smear layer before obturation of the canal. Smear layer removal is more commonly practiced by endodontists. It however is not been established as the standard of care, nor is its removal supported by clinical evidence.

Some studies have found that it is beneficial to retain the smear layer and not remove it prior to canal obturation. Drake *et al.* (1994) observed that the presence of an intact smear layer produced by endodontic instrumentation may prevent initial bacterial colonization of dentinal tubules. Their article showed a 10 fold reduction of colony-forming units for teeth with an intact smear layer compared to teeth with their smear layer removed. This suggested the presence of the smear layer prevents bacterial infiltration. The liability of exposing the dentinal tubules was reiterated by Pashley *et al.* (1981). They demonstrated that the smear layer could be removed after 15 seconds exposure to 6% citric acid, thus eliminating the protective function of the smear layer. Timpawat *et al.* (2001) found a significant difference between apical microleakage of extracted and obturated teeth with and without the smear layer. Teeth with an intact smear layer leaked less.

Clark-Holke *et al.* (2003) found the opposite result. They reported 0% leakage in teeth with the smear layer removed and 60% leakage in teeth with an intact smear through their apical foramen following endodontic instrumentation and obturation with gutta percha and sealer. Their findings suggest that an intact smear layer in the apical region leads to an inferior seal. The poor adaptation by the gutta percha and sealer was attributed to remaining debris that could include bacteria. Long-term evaluations are required when the smear layer is not removed due to its non-homogenous and non-fixed nature which subjects it to breakdown. It may slowly disintegrate or undergo removal by bacterial by-products and negatively influence the quality of the obturation and cement seal (Sen *et al.* 1995).

A systematic review of leakage studies by Shahraven *et al.* (2007) supported Clark-Holke *et al.* (2003) in finding the removal of the smear layer decreased leakage. More endodontists today believe it prudent to remove the smear layer. Reasons for this include enhancement of

disinfection of the canal by removing a potential source of bacterial infection and allowing better penetration of medicaments and sealers (Torabinejad *et al.* 2002 and Violich *et al.* 2010).

Methods to Remove the Smear Layer

Contemporary approaches to remove the smear layer employ chemicals, ultrasonics and lasers. These methods are neither universally accepted nor completely effective in the total removal of the layer (Violich and Chandler 2010). Irrigation with NaOCl removed pulpal remnants and predentin from canal walls but left behind a smear layer on the instrumented walls. When EDTA was used as an irrigant, the smear layer was demineralized but both pulpal remnants and predentin remained. The combination of NaOCl and EDTA completely removed pulpal remnants, predentin, and the smear layer (Baumgartner and Mader 1987).

Bystrom and Sundqvist (1985) reported that 15% EDTA used with 5% NaOCl was more efficient in removing bacteria from canals than NaOCl alone, possibly because bacteria were located within the smear layer. However, Robinson *et al.* (2012) found no significant difference in smear layer removal between irrigation of 17% EDTA or 6% NaOCl using Micro Computed Technology imaging. The later study used human mandibular molars, which display greater anatomical variation (isthmuses, protrusions and fins). The more complex anatomy of these teeth enhanced debris accumulation and may explain why differences were not found between the two irrigants. It may also explain the differences in results between this article and the previous study which used single rooted teeth.

A SEM study by McComb and Smith (1975) discovered that a commercially available EDTA called REDTA more effectively removed the smear layer when compared to NaOCL, NaOCL and hydrogen peroxide, RC-Prep and polyacrylic acid. Wu *et al.* (2012) found EDTA

significantly more effective than 20% citric acid, BioPure MTAD (a mixture of doxycycline, citric acid, and Tween 80), and SmearClear (another commercially available EDTA based canal irrigating solution). However, none of the four irrigants completely removed the smear layer in the apical third of the canal. Arslan *et al.* (2013) evaluated the apical regions of canals and documented they were cleaner when 15% EDTA was agitated with a laser for 20 seconds. In another study, continuous ultrasonic irrigation of 15% EDTA and 6% NaOCl led to significantly less debris in the apical third when compared to conventional needle irrigation using the same irrigants (Curtis *et al.* 2012).

Torabinejad *et al.* (2002) described the properties an irrigant or an intracanal medicament should possess to effectively disinfect the root canal system:

1. It should completely remove the smear layer.
2. It should disinfect the dentin and its tubules.
3. It should have sustained antibacterial effect after use.
4. It should allow the penetration of antimicrobial agents present in the solution into the dentinal tubules.
5. It should be nonantigenic, nontoxic, and noncarcinogenic to tissue cells surrounding the tooth.
6. It should have no adverse effects on the physical properties of exposed dentin.
7. It should have no adverse effect on the sealing ability of filling materials.
8. It should not discolor the tooth.

9. It should have convenient application.

10. It should be relatively inexpensive.

Irrigation with EDTA and NaOCl became an accepted smear layer removal system following the establishment of contact time and volume from *in vitro* SEM study evidence. Calt and Serper (2002) found that rinsing with 17% EDTA for no more than one minute removed the smear layer and minimized dentin erosion. Crumpton *et al.* (2005) discovered that 1ml of 17% EDTA in a single canal for one minute efficiently removed the smear layer with no benefit to using larger volumes of EDTA.

The removal of the smear layer is not without problems. More recently, Qian *et al.* (2011) reported that the common practice of using a one minute rinse with 17% EDTA followed by NaOCl potentially weakened the root by removing significantly more dentin than 17% EDTA alone. Removal of hydroxyl apatite by the EDTA exposed collagen fibrils leaving the amino acids susceptible to degradation by the NaOCl. Singh *et al.* (2009) found no significant differences in root dentin microhardness between 17% EDTA, EDTAC (ethylenediaminetetraacetic acid plus Cetavlon solution), BioPure MTAD (mixture of tetracycline isomer/ an acid/ a detergent) or RC-Prep (15% EDTA, 10% urea peroxide and glycol) irrigating solutions and lubricants.

Objective

To date, no prospective controlled in vivo study has been published evaluating the effectiveness of smear layer removal on healing in permanent teeth. Knowing the advantages and disadvantages of its removal on the final outcome will assist and guide practitioners in establishing their clinical protocol. The purpose of this continuing prospective, randomized,

double-blinded clinical trial is to compare the controlled use 17% EDTA to remove the smear layer against 0.9% saline as final irrigation solutions on endodontic outcomes of initial non-surgical root canal treated teeth. The study outcome, defined as healed or not healed, was based on clinical and radiographic findings at least one year following the completion of treatment. Secondarily, the influence of covariant factors on endodontic outcomes was analyzed. It was hypothesized that removing the smear layer would result in a better endodontic outcome.

Chapter III: Materials and Methods

[This section was referenced from WRNMMC IRBNet # 352491, "The Effect of Smear Layer Removal on Endodontic Outcomes protocol" (Version 8 10 Sep 14)].

The Naval Postgraduate Dental School Endodontics Dept. is a referral service drawing eligible beneficiaries from the National Capitol Region. Prior to any performing treatment, all patients receive a thorough diagnostic examination using the Subjective, Objective, Assessment, and Plan (S.O.A.P) format. Diagnoses made capture all appropriate measurements using an armamentarium conforming to the "standard of care" for the endodontics specialty. Patients considered for this study met the following study inclusion criteria:

- Eligible beneficiaries eighteen years or older
- The ability to make an informed decision and consent to participate
- Patient classification of I or II by the American Society of Anesthesiology (ASA)

  ASA physical status I, a normal healthy patient or ASA physical status II, a patient with mild systemic disease (well-controlled hypertension) and no functional limitations such as smoker
- One visit, initial, non-surgical endodontic treatment
- Any pulpal diagnosis and an apical diagnosis of normal apical tissues, symptomatic apical periodontitis, asymptomatic apical periodontitis, chronic apical abscess or condensing osteomyelitis
- Presence or absence of a periapical radiolucency
- Presence of preoperative pain or pain-free
- Return to the clinic for an evaluation appointment 1-year following treatment

Patients were excluded from the study if they presented with any of the following:

- Under eighteen years of age
- Unable or not willing to participate
- Pregnancy or a ASA classification of III or greater

  ASA Physical Status III - A patient with severe systemic disease, ASA Physical Status IV- A patient with severe systemic disease that is a constant threat to life, ASA Physical Status V- A moribund patient who is not expected to survive without the operation, ASA Physical Status VI - A declared brain-dead patient whose organs are being removed for donor purposes
- Existing nonsurgical root canal treatment or a previously initiated but uncompleted root canal treatment
- Canals medicated with calcium hydroxide [$Ca(OH)_2$]or any other dental medicament
- Allergy to any medication or dental material used in study including; 17% EDTA, 6% NaOCl, latex or gutta percha
- Patients presenting with periodontal disease or acute apical abscess
- Patients currently taking antibiotics

If a patient expressed an interest to participate, a principal or associate investigator, not providing their care, was contacted to read a script explaining the study. Informed consent was obtained from all study participants meeting inclusion criteria. Once enrolled, each subject was assigned a unique study identification number established by their chronologic order of enrollment and randomly assigned into one of two groups which differed only in the application of the final irrigation solution rinse. Clinical care was provided by Endodontic residents at the

Naval Postgraduate Dental School. The following standardized treatment procedure was employed for all study subjects:

- A Rinn XCP (Dentsply International, York, PA) holding guide was used to fabricate a positioning device by applying Blu-Mousse (Parkell, Edgewood, NJ) to both sides of the bite tab. Once set, this customized device allowed duplication of the vertical and horizontal angles for all radiographs.
- Two pre-operative periapical radiographs were taken. The first radiograph was made with the x-ray beam perpendicular to the long axis of the tooth and perpendicular to the floor. The second radiograph was taken with the x-ray tube head shifted 15° to the mesial of the tooth in the horizontal plane. The vertical angulation was maintained at 0°. If traditional radiographic film was used, one of the radiographs remained in the patient's dental record. The second radiograph was kept in the subjects study file folder.
- Dental anesthesia was administered
- The operative area surrounding the tooth was isolated using rubber dam and a pre-packaged caulking adhesive, Oraseal® (Ultradent Products, South Jordan, UT)
- A #2 round or #557 carbide bur (Henry Schein, Melville, NY) and an EndoZ bur (Dentsply Maillefer, Tulsa, OK) were used to obtain straight-line access into pulp chamber
- All treatment was carried out with the aid of a dental operating microscope (DOM). Additional dental radiographs were taken as required for good endodontic treatment, but no more than necessary.
- Canal orifice locations were established with the aid of an endodontic explorer and ultrasonics when applicable

- 0.9% saline irrigation was used as required
- Patency length was established with a 0.02 taper #10 stainless steel FlexoFile® (Dentsply Maillefer, Tulsa, OK) in all root canals using a Root ZX® (J Morita, Irvine, CA) apex locator
- The working length was set 1 mm short of patency length and confirmed with two periapical radiographs
- Coronal flaring of a canal was created using # 2, 3, 4 Gates Glidden drills (SybronEndo Corporation, Orange, CA)
- Instrumentation of the apical one third of the canal was performed using 0.02 taper #10, #15, #20 FlexoFile® stainless steel Flex-O file to working length while irrigating with 0.25 ml 6% NaOCl for a total NaOCl irrigant volume of 0.75 ml. All irrigation was delivered with a 1 inch 30 gauge Max-i-probe syringe (Dentsply Maillefer, Tulsa, OK)
- Crown down rotary instrumentation was accomplished using a 0.04 Profile (Dentsply Maillefer, Tulsa, OK) to a master apical file size of 0.04 taper #40

  Crown down (CD) instrumentation emphasizes the sequential cleansing and shaping of the coronal (1st), middle (2nd), and apical thirds (last) (Marshall and Pappin, 1980). The CD approach creates coronal taper while minimizing file contact area to reduce torsional forces on the file. This "crown-down pressureless technique" involves early canal flaring with Gates Glidden drills or orifice shapers, followed by the incremental removal of canal contents and dentin proceeding from the canal orifice to the working length. Straight files are used in a larger to smaller sequence with a reaming motion and no apical pressure once the instrument begins to bind in the canal.

- Canal recapitulation was performed using a 0.02 taper #10 FlexoFile to working length

while irrigating with 0.25 ml 6% NaOCl between all file sizes for a total intraoperative irrigation volume of 2 ml

- The canals were dried with sterile paper points (Henry Schein, Melville, NY)

Canal instrumentation was completed at this point in the procedure and the provider was given one of two final irrigating solutions in a 3ml syringe with a side vented, one-inch, 30 gauge Max-i-probe tip (Dentsply Maillefer, Tulsa, OK) labeled irrigant A or irrigant B. The syringe contained either 1ml of 17% EDTA or 1ml of 0.9% sterile saline. The 1ml volume was delivered to each instrumented canal over 1 minute period. Patients and providers were blinded as to which final irrigant was used and both irrigation techniques fell under the existing "Standard of Care" for root canal therapy. Treatment was resumed with all subjects receiving the following obturating procedure:

- The canals were dried with sterile paper points (Henry Schein, Melville, NY)
- A final rinse with 3ml 6% NaOCl per canal was administered
- The canals were dried with sterile paper points (Henry Schein, Melville, NY)
- A System B® (SybronEndo, Orange, CA) plugger was selected that best matched individual canal size and taper
- The plugger was pre-fit to binding point (5-7 mm short of length) and marked with a rubber stopper
- A 0.04 taper #40 master gutta percha cone (Diadent, Burnaby, BC, Canada) was placed in the canal to confirm the cone seated to working length
- The canal wall and master cone was coated with a mixture of eugenol and Grossman Type 801 Root Canal Cement Powder (Roth International LTD, Chicago, IL), the sealer was delivered into the canal using a lentulospiral (Dentsply Maillefer, Tulsa, OK)

- The System B at was set 200°C at a power setting of 10
- The gutta-percha cone seared at orifice with the heated plugger, all excess gutta-percha was removed
- The activated plugger was driven to within 3mm of binding point (over 1.5-3.5 seconds), it was deactivated, apical pressure was maintained until plugger reached binding point
- Apical pressure at binding point was maintained for 10 seconds
- Under continued apical pressure, the System B was activated for 1 second, the plugger was quickly withdrawn
- The plugger was then used to confirm that apical mass of gutta-percha had not been dislodged
- The canal was backfilled using an Obtura II™ (Obtura Spartan, Earth City, MO) set at 200°C
- The chamber was cleaned with an alcohol-soaked cotton pellet
- The access chamber was temporized with a sterile cotton pellet and Fuji IX GP® (GC America Inc., Alsip, IL) packable glass-ionomer cement
- Two immediate post-operative periapical radiographs were taken with duplicate film using the subject's custom positioning device. The first radiograph was made with the x-ray beam perpendicular to the long axis of the tooth and perpendicular to the floor. A second radiograph was taken with the x-ray tube head shifted 15° to the mesial of the tooth in the horizontal plane. The vertical angulation remained at 0°. One set of radiographs remained in the patient's dental record. The second set was placed in the subject's study file folder.

All data were recorded on IRB approved forms. This included patient information (Appendix

A), preoperative (Appendix B), intra-operative (Appendix C) and follow-up (Appendix D) data. The subject's unique identification number was used to identify a subject's file folder, data collection sheets and radiographs. All subject files were maintained in a secure location when not in use.

Follow-up Evaluation

A standardized clinical and radiographic examination was completed for all subjects at least one year after nonsurgical root canal treatment was completed. The clinical evaluation included a review of the medical history and subjective assessment of pain (0-10 scale) for the tooth that received treatment. The tissue in the area was palpated to assess for swelling, tenderness and examined for the presence of a sinus tract. The tooth was percussed and tested for cold sensitivity or mobility. It was also electric pulp tested and sulcular probing depths captured. The follow-up data was recorded in Appendix D and included pulpal and apical diagnoses and two periapical radiographs taken with duplicate film utilizing the subject's custom positioning device.

Data Analysis

A power analysis established a sample size based on an 80% overall healing rate obtained from previously published outcome studies. In order to estimate the true rate of healing to within 5 percentage points, 200 teeth per group will be required.

Healing was evaluated both clinically and radiographically. The study outcome, ether healed or non-healed was based on clinical symptoms and radiographic data collected at the 1-year follow-up examination The presence of swelling, an unresolved sinus tract or sensitivity to palpation or percussion were clinical indicators that the tooth was non-healed. The periapical radiographs were projected in random order onto a screen in a dark room and individually scored using the Periapical Index (PAI) (Orstavik, et al. 1986) by 3 calibrated, board-certified, endodontists. PAI scores of 1 and 2 indicated healed while PAI scores of 3, 4 and 5 were non-healed. In cases of disagreement, a

consensus between the examiners determined the final PAI score of the tooth. A tooth had an outcome of healed only if it was both clinically asymptomatic and had a radiographic PAI score of 1 or 2. This study used strict outcome scoring criteria. The data were analyzed using the Fisher's Exact Chi Square and odds ratio tests.

Chapter IV: Results

At the time of this interim analysis, two hundred thirteen subjects were enrolled in the study. One hundred eighty two had received treatment at least one year prior and were eligible for follow-up examinations. Of those eligible, one hundred fifty seven presented to the clinic yielding a recall rate of 86%. Twenty six of these subjects were removed from the analysis, 12 teeth were verified as extracted and 14 subjects required multiple visits to complete treatment which was a deviation from protocol. The one hundred thirty one teeth that were treated under this study's strict protocol were then analyzed (Table 1.).

Status	# Subjects
Enrolled	213
Eligible for follow-up	182
Recalled	157
Recall Rate	86%
Extracted	12
Protocol Deviation	14
Analyzed	131
Symptomatic	2

Table 1.

The sample demographics were 74% male (97) and 26% female (34) and ranged in age from 18 to 75 years. The proportion of single rooted teeth was 43% (56 teeth) and the remaining 57% (75 teeth) had multiple roots. The sixty eight teeth in the 17% EDTA group (smear layer removed) had a healed rate of 47% while 53% did not heal. The 0.9% saline group (smear layer not removed) consisted of sixty three teeth and had a healed rate of 62% while 38% did not heal. Fishers Chi-

square analysis revealed no significant difference (p=0.114) between the two groups (Table 2.) The proportion healed was independent of the saline or EDTA irrigant used. It should be noted that only 2 subjects reported clinical symptoms at the follow-up evaluation. The remaining subjects were classified as non-healed based on their follow-up periapical radiograph.

Seventy percent (92) of the teeth were vital and 30% (39teeth) non-vital. Healed rates of single versus multiple rooted teeth were not significant (p=0.063). Sixty four percent of the single rooted teeth and 48% of the multiple rooted teeth healed. If the 14 subjects requiring multiple appointments to complete their treatment had been included at the 12 month or greater follow up examination, 145 (92%) teeth would be classified as clinically asymptomatic.

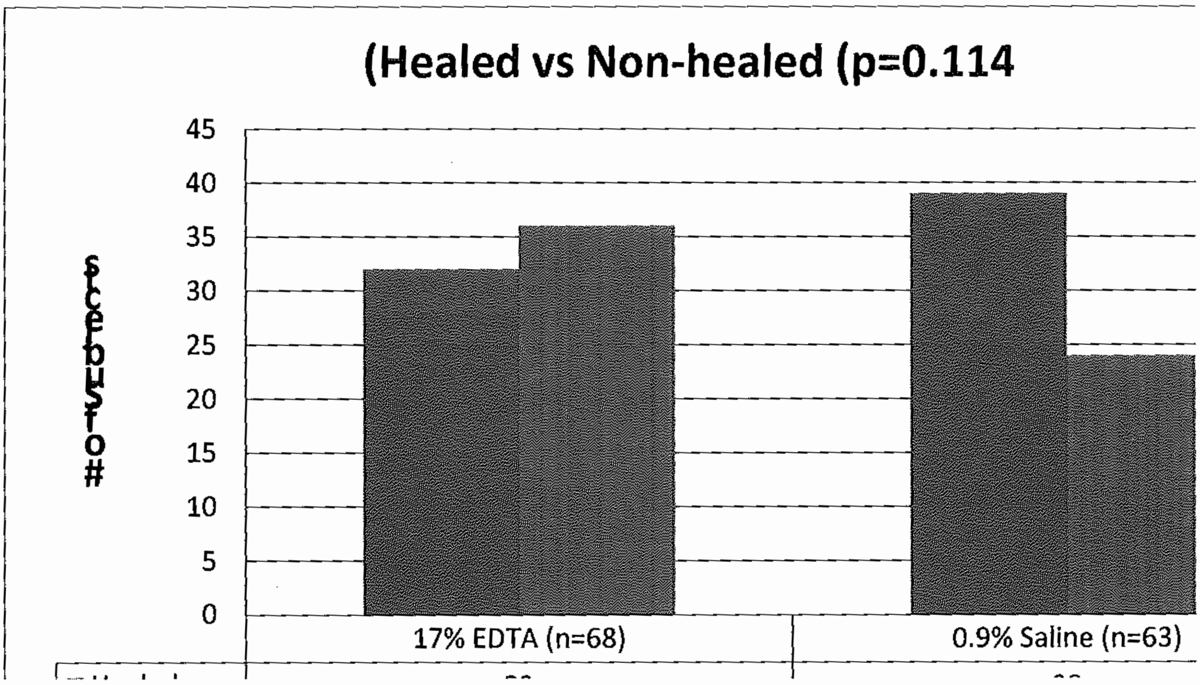

Table 2.

The secondary objective of the study was to evaluate possible covariate influences on endodontic outcomes. The following covariate factors were analyzed: gender, tooth position, tooth type, caries, cold sensitivity, diabetes, HTN, EPT, pain, percussion, pulpal diagnosis, apical

diagnosis, restoration, bleeding on probing, and intra-orifice barrier. None of the covariates were found to significantly effect endodontic outcome.

Chapter V: Discussion

The evidence to date remains unclear concerning the effect of smear layer on endodontic outcome. The literature supports both leaving and removing it just prior to canal obturation. And, a majority of clinicians today favor removing the smear layer based on a belief that "cleaner is better". This study hypothesized that removing the smear layer would result in better endodontic outcomes. It has been demonstrated that removal improves the adaptation of root filling materials (Shahraven *et al.* 2007) and increases the elimination of debris and bacteria (Soares *et al.* 2010). Other reasons cited to remove smear layer include increased medicament effectiveness on infected dentin (Orstavik and Haapasalo 1990) and better medicament diffusion (Torabinejad *et al.* 2002). Bystrom and Sundqvist (1985) found the bacteria that survived canal instrumentation and irrigation with 0.5% or 5% NaOCl rapidly multiplied. During multi-appointment visits it is recommend to place an intracanal medicament such as calcium hydroxide in the empty canal between appointments to inhibit bacterial repopulation. A significant difference was found regarding the amount of calcium detected on instrumented root surfaces following calcium hydroxide placement into the root canals of teeth with intact versus removed smear layer. Foster *et al.* (1993) reported a greater penetration of calcium hydroxide to the exterior root surface when the smear layer was removed. This suggested presence of the smear layer inhibited calcium hydroxide from entering the dentinal tubules. However, a later study found that an intact smear layer only delayed the effect of calcium hydroxide and did not prevent its ability to kill bacteria (Orstavik and Haapasalo 1990). Surveys have demonstrated an increasing trend among endodontists to routinely remove the smear layer during non-surgical root canal treatment (Moss *et al.* 2001; Dutner *et al.* 2012). Finally, there is the empirical belief within the Endodntic community that cleaner is better.

This study conversely found no difference in endodontic outcome when removing or not removing the smear layer prior to obturation. There is ample evidence to support leaving an intact smear layer prior to obturating the root canal. This includes a decrease in number of bacteria found in dentinal tubules following canal instrumentation (Drake *et al.* 1994), increased dentinal erosion when NaOCl is used following an EDTA rinse (Qian *et al.* 2011), a decrease in apical microleakage when the smear layer is left intact (Timpawat *et al.* 2001), and a decrease in cost and procedure time have been reported. Kakehashi, Stanley and Fitzgerald (1965) definitively demonstrated that bacteria within the canal system were responsible for apical pathosis. Treatment success is directly related to removal of this etiologic agent, not the presence or absence of the smear layer. Clegg *et al.* (2006) demonstrated that the 6% NaOCl used in this study is not only effective in eradicating planktonic bacteria but also destroys the biofilm environment that protects bacteria. In 2014, Ferrer-Luque and Bejarano found that rotary instrumentation itself significantly reduced bacterial load independent of either distilled water or NaOCl use.

Although clinicians actively attempt to remove the smear layer using chemo-mechanical techniques, the limited access and complexity of the root canal system inhibits its complete elimination. Gambarini and Laszkiewicz (2002) observed a smear layer using SEM following a final irrigation with ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCL). The layer was unevenly distributed within the canal with the least amount was found in the coronal third and the greatest amount in the apical third. Significant differences were found between all portions of the canal with the greatest difference was between the coronal and apical thirds. Additionally, Peters and Schonenberger (2001) found that 37% of the canal surface remained untouched, i.e. no smear layer is created, using the same ProFile 0.04 taper rotary files

used in this study. Since the smear layer can never be completely removed, these finding support that attempts to remove the smear layer may not significantly affect outcome.

Covariate factors have been shown to influence healing in other studies. Previous interim analyses found significant differences in healing between medical conditions and single and multi-rooted teeth. The enrollment of additional subjects has rendered the influence of these covariates non-significant on healing in this current analysis. A tooth with a normal pulp diagnosis and no history of bacterial infection would be expected to have a greater healed rate. A Chi square analysis found a highly significant difference between subjects with a pre-operative normal pulp diagnosis (n=30) in relation to smear layer removed and smear layer not removed vs. outcome (p=0.009, Table 3.). In this *in vivo* study, the odds of healing increased by 10 times for individuals exposed to 0.9% saline compared to 17% EDTA, suggesting that outcome of endodontic treatment was dependent on the irrigant used. This finding is consistent with the study performed on extracted teeth by Drake *et al.* (1994). Following instrumentation, they compared a final rinse of 20ml saline rinse to the combination of 10ml 17% EDTA and 10ml 2.5% NaOCl. They reported the presence of an intact smear layer produced by endodontic instrumentation prevented initial bacterial colonization of dentinal tubules.

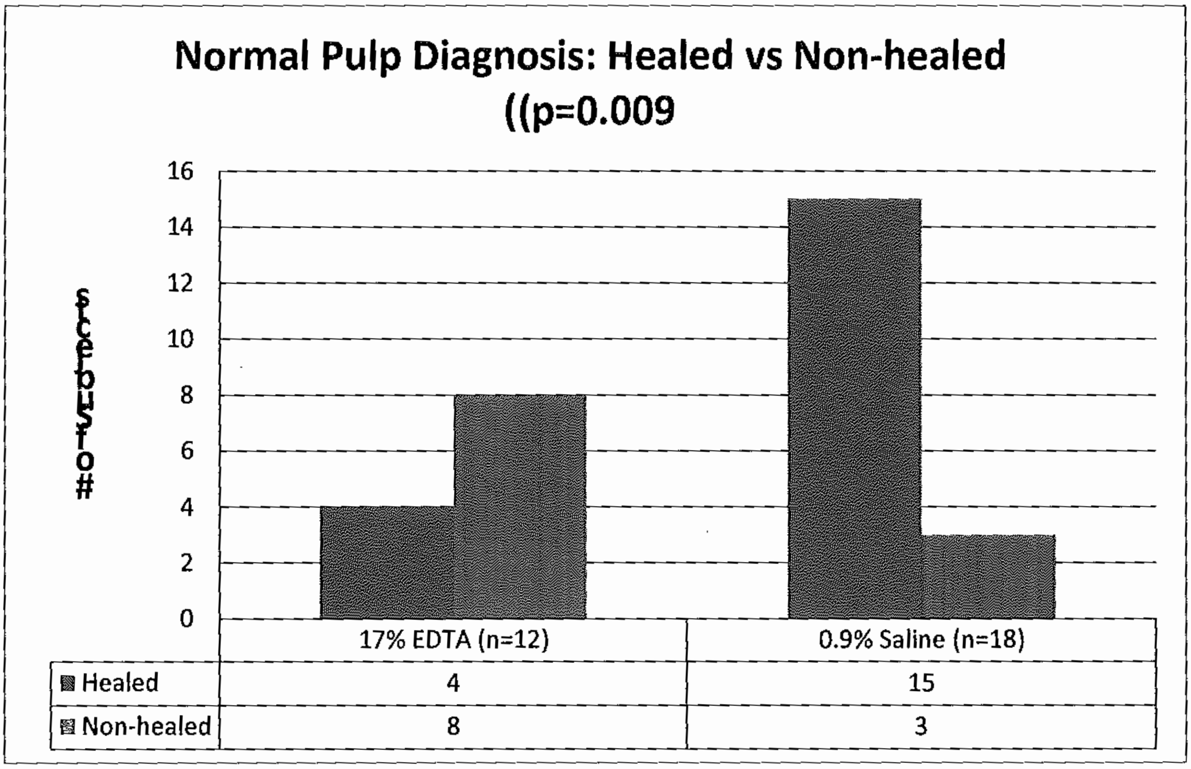

Table 3.

Irrigant Used

Twenty six teeth were removed from this analysis. Although 12 were extracted, anecdotal evidence indicated the majority of these were for prosthodontic reasons, not endodontic failure. Fourteen subjects required multiple visits to complete their treatment. Sjögren *et al.* (1991) recommended placing calcium hydroxide into instrumented canals to reduce the bacterial load between appointments. Molander *et al.* (2007) however found no significant difference in healing outcomes between single-visit vs. two-visit treatments when using calcium hydroxide as an intracanal medicament for one week. This suggests there may be no benefit in using this antimicrobial agent and that obturating the canal during the initial appointment yields similar clinical outcomes as performing the procedure in two appointments. Alternatively, Safavi *et al.* (1990) reported the persistence of enterococci after exposure to calcium hydroxide. To limit the effect of

this confounding variable the study inclusion criteria limited treatment to one visit.

This is the first clinical trial investigating the effect of smear layer removal on endodontic outcomes in permanent teeth. The only other studies that have investigated the presence or absence of smear layer on outcome were both performed on primary teeth. In 2011 Tannure *et al.* found no significant difference in the success of pulpectomies after removing or not removing the smear layer. However, Barcelos *et al.* (2012) showed increased success from 70% to 91% when the smear layer was removed from primary teeth during a pulpectomy. The study used a citric acid solution to remove the smear layer, performed treatment over two visits, used a camphorated paramonochlorophenol interim intracanal medicament, irrigated with 2.5% NaOCl, obturated the primary canal with zinc oxide-eugenol paste, and evaluated the outcome after twenty four months. The use of deciduous teeth and differences in study methodologies make any comparisons to this study inaccurate.

Study limitations include the follow-up duration and the strict definition of success. Ng *et al.* (2011) found an increase in healed rates from 72% to 91% by increasing the follow up duration from one to two years. Friedman (2002) found signs of healing at the one year mark but stated three to four years may be necessary to observe healing. This study did not calculate a functional success rate, only healed rates using both clinical symptoms and strict PAI criteria.

Chapter VI: Conclusion

The interim analysis results of this prospective randomized double-blinded clinical trial indicates that removal of the smear layer does not affect the endodontic outcome of initial non-surgical root canal treatment in permanent teeth.

Appendix A

Patient #: ________

Smear Layer Patient Information and Preoperative Data

Name: ______________________________ Rank/rate: __________ Service: _______

DOB: ___________ Last 4: ________ Gender: M / F Tooth #: _______

Work address: ____

Home address: ____

Permanent address: ____

Phone # (primary): _________________ Phone # (secondary): ________________________

Work e-mail: _______________________ Personal e-mail: _________________________

Appendix B

Subject #: ________

Smear Layer Preoperative Data

Tooth type: single root multiple root

Does patient have any of the following conditions (circle):

Hypertension Smoker Coronary Heart Disease Diabetes Type: ____

Symptoms: Y/N

___ Can locate pain by quadrant

___Pain (0-10) (Y/N)
___Can locate pain by quadrant (Y/N)
___Can locate pain by tooth (Y/N)
___Tooth #
___/80 Electric pulp tester
___Palpation sensitivity
___Sinus tract (Y/N)
___Swelling (Y/N)
___History of Ortho tx (Y/N)
___History of external resorption (Y/N)
___Post (Y/N)
___Caries

___Cold sensitivity (R/NL; R/I; NR)
___Percussion sensitivity (S/NS)
___Mobility (Miller's Class)
___Bleeding on probing
___History of bleaching (Y/N)
___History of internal resorption (Y/N)
___Retreatment (Y/N)
___Surgical/nonsurgical treatment
___Open margin (Y/N)
___Restoration present (Y/N)
___Duration of symptoms (mos.)

PPD (mm)	Buccal	Lingual
Mesial		
Mid		
Distal		

Preoperative Radiographic findings:

Intact lamina dura (Y/N) Radiolucency (Y/N) Size___x___mm

Preoperative Diagnosis:

Pulpal:
___Normal pulp
___Reversible pulpitis
___Symptomatic irreversible pulpitis
___Asymptomatic irreversible pulpitis
___Pulp necrosis
___Previously treated
___Previously initiated therapy

Apical:
___Normal apical tissues
___Symptomatic apical periodontitis
___Asymptomatic apical periodontitis
___Acute apical abscess
___Chronic apical abscess
___Condensing osteitis
___Lesion of non endodontic origin

Appendix C

Subject #: ________

Smear Layer Intraoperative Data

Working length established using electronic apex locator: Y/N

Patency Achieved:

____ Canal Y/N
____ Canal Y/N
____ Canal Y/N
____ Canal Y/N
____ Canal Y/N

Type of Irrigants used (ml):

$Ca(OH)_2$ used as interappointment medicament: Y/N

Procedural complications: Y/N Type:

Intraorifice barrier placed: Y/N type:

Number of treatment sessions: single multiple

Obturation fill length:

____ Flush (≤2 mm from apex)
____ Overextension (beyond apex)
____ Underextension (>2 mm short of apex)

Post treatment Diagnosis

Pulpal:

____ Normal pulp
____ Reversible pulpitis
____ Asymptomatic irreversible pulpitis
____ Symptomatic irreversible pulpitis
____ Pulp necrosis
____ Previously treated
____ Previously initiated therapy

Apical:

____ Normal apical tissues
____ Symptomatic apical periodontitis
____ Asymptomatic apical periodontitis
____ Acute apical abscess
____ Chronic apical abscess
____ Condensing osteitis
____ Lesion of non endodontic origin

Date of Treatment Completion: ________

EVALUATOR USE ONLY

Final treatment radiographic Periapical Index (PAI) score: 1 2 3 4 5

Appendix D

Subject #: ________

Smear Layer Follow-up Data

Date of follow-up evaluation: ____________

Does patient have any of the following conditions (Circle):

Hypertension   Smoker   Coronary Heart Disease (CHD)   Diabetes Type: ____

Symptoms: Y/N

____ Pain (0-10)

____ EPT

____ Palpation sensitivity (S/NS)

____ Sinus tract (Y/N)

____ Swelling (Y/N)

____ Time Elapsed Between Initial Tx and Permanent Restoration

____ Duration of symptoms

____ Cold sensitivity (R/NL, R/L, NR)

____ Percussion sensitivity (S/NS)

____ Mobility (Miller's Classification)

____ Periodontal Screening Record (PSR)

____ Bleeding on probing

PPD (mm)	Buccal	Lingual
Mesial		
Mid		
Distal		

Follow-up Radiographic findings:

Intact lamina dura Y/N   Radiolucency (Y/N) Size ____ x ____ mm

Follow up diagnosis: (Apical)

____ Normal apical tissues

____ Symptomatic apical periodontitis

____ Asymptomatic apical periodontitis

____ Acute apical abscess

____ Chronic apical abscess

____ Condensing osteitis

____ Lesion of non endodontic origin

Caries present? Y/N

Permanent coronal restoration present? Y/N

Intracanal post present? Y/N

Open Margin Y/N

Surgical or Nonsurgical Treatment

EVALUATOR USE ONLY

Final treatment radiographic Periapical Index (PAI) score: 1 2 3 4 5

REFERENCES

1. Arslan H, Ayranci L, Karatas E, Topcuoglu H, Yavuz M, Kesim B. (2013). Effect of agitation of EDTA with 808-nanometer diode laser on removal of smear layer. *Journal of Endodontics,* 39, 1589-92.

2. Barcelos R, Tannure P, Gleiser R, Luiz R, Primo L. (2012). The influence of smear layer removal on primary tooth pulpectomy outcome: a 24-month, double-blind, randomized, and controlled clinical trial evaluation. *International Journal of Paediatric Dentistry,* 22 (5), 369-81.

3. Baumgartner J, Mader C. (1987). A scanning electron microscope evaluation of four root canal regimens. *Journal of Endodontics*, 13 (4), 147-157.

4. Bystrom A, Sundqvist G. (1985). The antibacterial effect of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *International Endodontic Journal*, 18 (6), 35-40.

5. Calt S, Serper A. (2002). Time-dependent effects of EDTA on dentin structures. *Journal of Endodontics,* 28, 17–9.

6. Clark-Holke D, Drake D, Walton R, Rivera E, Guthmiller J. (2003). Bacterial penetration through canals of endodontically treated teeth in the presence or absence of the smear layer. *Journal of Dentistry,* 31, 275-81.

7. Clegg M, Vertucci F, Walker C, Belanger M, Britto L (2006). The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. *Journal of Endodontics,* 32(5), 434-7.

8. Crumpton B, Goodell G, McClanahan S. (2005). Effects on smear layer and debris removal with varying volumes of 17% REDTA after rotary instrumentation. *Journal of Endodontics,* 31 (7), 536-8.

9. Curtis T, Sedgley C. (2012). Comparison of a continuous ultrasonic irrigation device and conventional needle irrigation in the removal of root canal debris. *Journal of Endodontics*, 38, 1261-4.

10. Drake D, Wiemann A, Rivera E, Walton R. (1994). Bacterial Retention in Canal Walls In Vitro: Effect of Smear Layer. *Journal of Endodontics*, 20 (2), 78-82.

11. Dutner J, Mines P, Anderson A. (2012). Irrigation Trends among American Association of Endodontists Members: A Web-based Survey. *Journal of Endodontics*, 38 (1), 37-40.

12. Eick J, Wilko R, Anderson C, Sorensen S. (1970). Scanning electron microscopy of cut tooth surfaces and identification of debris by use of the electron microprobe. *Journal of Dental Research,* 49, 1359-68.

13. Ferrer-Luque C, Bejarano I. (2014). *International Endodontic Journal*, 47, 380-6.

14. Foster K, Kulild J, Weller R. (1993). Effect of smear layer removal on the diffusion of calcium hydroxide through radicular dentin. *Journal of Endodontics,* 19 (3), 136-40.

15. Friedman S. (2002). Prognosis of initial endodontic therapy. *Endodontic Topics*, 2, 59-88.

16. Gambarini G, Laszkiewicz J. (2002). A scanning electron microscopic study of debris and smear layer remaining following use of GT rotary instruments. *International Endodontic Journal,* 35, 422-27.

17. Kakehasi S, Stanley H, Fitzgerald R. (1965). The Effects of Surgical Exposure of Dental Pulps in Germ Free and Conventional Laboratory Rats. *Oral Surgery, Oral Medicine, and Oral Pathology*, 20 (3), 340-9.

18. Mader C, Baumgartner J, Peters D (1984) Scanning electron microscopic investigation of the smeared layer on root canal walls. *Journal of Endodontics,* 10 (10), 477–83.

19. Marshall F, Pappin J. (1980). A crown-down pressureless preparation root canal enlargement technique [manuel]. Portland, Ore: Oregon Health Sciences University

20. McComb D, Smith D. (1975). A preliminary scanning electron microscopic study of root canals after endodontic procedures. *Journal of Endodontics*, 1, 238-42.

21. Mjor A, Nordahl I. (1996). The density and branching of dentinal tubules in human teeth. *Archieves of Oral Biology,* 41 (5), 401-12.

22. Molander A, Warfvinge J, Reit C, Kvist T. (2007). Clinical and radiographic evaluation of one- and two-visit endodontic treatment of asymptomatic necrotic teeth with apical periodontitis: A randomized clinical trial. *Journal of Endodontics,* 33, 1145-48.

23. Moss D, Allemang J, Johnson J. (2001). Philosophies and Practices Regarding the Management of the Endodontic Smear Layer: Results from Two Surveys. *Journal of Endodontics,* 27 (8), 537-9.

24. Ng Y, Mann V, Gulabivala K. (2011). A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. *International Endodontic Journal*, 44, 583-609.

25. Orstavik D, Haapasalo M. (1990). Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. *Endododontics and Dental Traumatology*, 6 (9), 142-149.

26. Orstavik D, Kerekes K, Eriksen H. (1986). The periapical index: a scoring system for radiographic assessment of apical periodontitis. *Endododontics and Dental Traumatology,* 2, 20-34.

27. Pashley D. (1992). Smear layer: overview of structure and function. *Proceedings of the Finnish Dental Society*, 88 (Suppl 1), 215-224.

28. Pashley D, Michelich V, Kehl T. (1981). Dentin permeability: effects of smear layer removal. *Journal of prosthetic dentistry*, 46, 531-7.

29. Peters O, Schonenberger K, Laib A. (2001). Effects of four Ni-Ti preparation techniques on root canal geometry assessed by micro computed tomography. *International Endodontic Journal,* 34, 221-30.

30. Qian W, Shen Y, Haapasalo M. (2011). Quantitative Analysis of the Effect of Irrigant Solution Sequences on Dentin Erosion. *Journal of Endodontics*, 37 (10), 1437-1441.

31. Robinson J, Lumley P, Claridge E, Cooper P, Grover L, Williams R, Walmsley A. (2012). An analytical Micro CT methodology for quantifying inorganic dentine debris following internal tooth preparation. *Journal of Dentistry,* 40, 999-1005.

32. Safavi K, Spangberg L, Langeland K. (1990). Root canal dentinal tubule disinfection. *Journal of Endodontics,* 16 (5), 207-210.

33. Sen B, Wesselink P, Turkun M. (1995). The Smear layer: a phenomenon in root canal therapy. *International Endododontic Journal*, 28 (3), 141-148.

34. Shahravan A, Haghdoost A, Adl A, Rahimi H, Shadifar F. (2007). Effect of smear layer on sealing ability of canal obturation: A systematic review and meta-analysis. *Journal of Endodontics,* 33, 96-105.

35. Singh S, Acharya S, Ballal V, Rijesh M. (2009). Evaluation of the effect of EDTA, EDTAC, RC-Prep and BioPure MTAD on the microhardness of root canal dentine- An in vitro study. *Endodontology,* 21 (2), 35-41.

36. Soares J, de Carvalho M, Santos S, Mendoça R, Ribeiro-Sobrinho A, Brito-Júnior M, Malgahães P, Santos M, Farias L (2010). Effectiveness of chemomechanical preparation with alternating use of sodium hypochlorite and EDTA in eliminating intracanal *enterococcus faecalis* biofilm. *Journal of Endodontics,* 36 (5), 894-8.

37. Sjögren U, Figdor D, Spångberg L, Sundqvist G. (1991). The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. *International Endodontic Journal*, 24, 119-125.

38. Tannure P, Azevedo C, Barcelos R, Gleiser R, Primo L. (2011). Long-term outcomes of primary tooth pulpectomy with and without smear layer removal: a randomized split-mouth clinical trial. *Pediatric Dentistry,* 33 (4), 316-20.

39. Timpawat S, Vongsavan N, Messer H. (2001). Effect of removal of the smear layer on apical microleakage. *Journal of Endodontics,* 27 (5), 351-3.

40. Torabinejad M, Handysides R, Khademi A, Bakland L. (2002). Clinical implications of the smear layer in endodontics: A review. *Oral Surgery Oral Medicine Oral Pathology,* 94 (6), 658-66.

41. Violich DR, Chandler NP. (2010) The smear layer in endodontics – a review, *International Endodontic Journal,* 43, 2-15.

42. Wu L, Mu Y, Deng X, Zhang S, Zhou D. (2012). Comparison of the effect of four decalcifying agents combined with 60 °C 3% sodium hypochlorite on smear layer removal. *Journal of Endodontics,* 38, 381-4.